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Dynamics of Rainbow Trout Bacterial Flora during Yersinia Ruckeri-Induced Enterosepticemia

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During a Yersinia-ruckeri-induced epidemic, we studied the bacterial flora harboured by the rainbow trout, Salmo gairdneri Richardson. Following antibiotic treatment with Apurone+, the micro-organism disappeared from samples that were systematically collected from the digestive compartments, kidneys, spleen, liver and muscle tissue. The pathogen re-emerged eleven days after the end of treatment but was unattended by any particular clinical signs and gradually became the dominant taxon in the flora found in the various samples (Table 1).

Identification of the strains that were isolated in the dominant flora showed that only one bacterial taxon, i.e., Hafnia alvei, was present on a permanent basis in the digestive compartments or other internal organs. The other bacterial species we identified included Aeromonas, Pseudomonas, Corynebacteria and several enterobacterial species.

+ Apurone = Flumequine (Riker Laboratories, avenue du 11 novembre, Pithiviers)

On the basis of a bacterial count on a polyvalent agar culture medium and a medium specifically designed for Gram-negative bacilli, it was found that results fell into one of two groups, i.e., findings for the digestive compartments and findings for samples from all other organs (Figures 1 and 2). The flora in the three digestive compartments varied little in quantitative terms during the course of the study, except for a dip in numbers on the 35th day. This drop was directly related to a fast imposed on the subjects because of piscicultural procedures. Once food was distributed again, the floral count returned to normal.

In the other organs (kidneys, spleen, liver and muscle tissue), there was a quantitative variation in the floral count with peak values recorded on the 11th, 21st-25th and 35th days. The first two peaks were associated with a rise in outside temperatures and the third corresponded to fish breeding procedures, i.e., sorting operations. They were symptomatic of stress in the fish. The floral count never exceeded 10^6 bacteria per gram of analyzed organ tissue.

Discussion

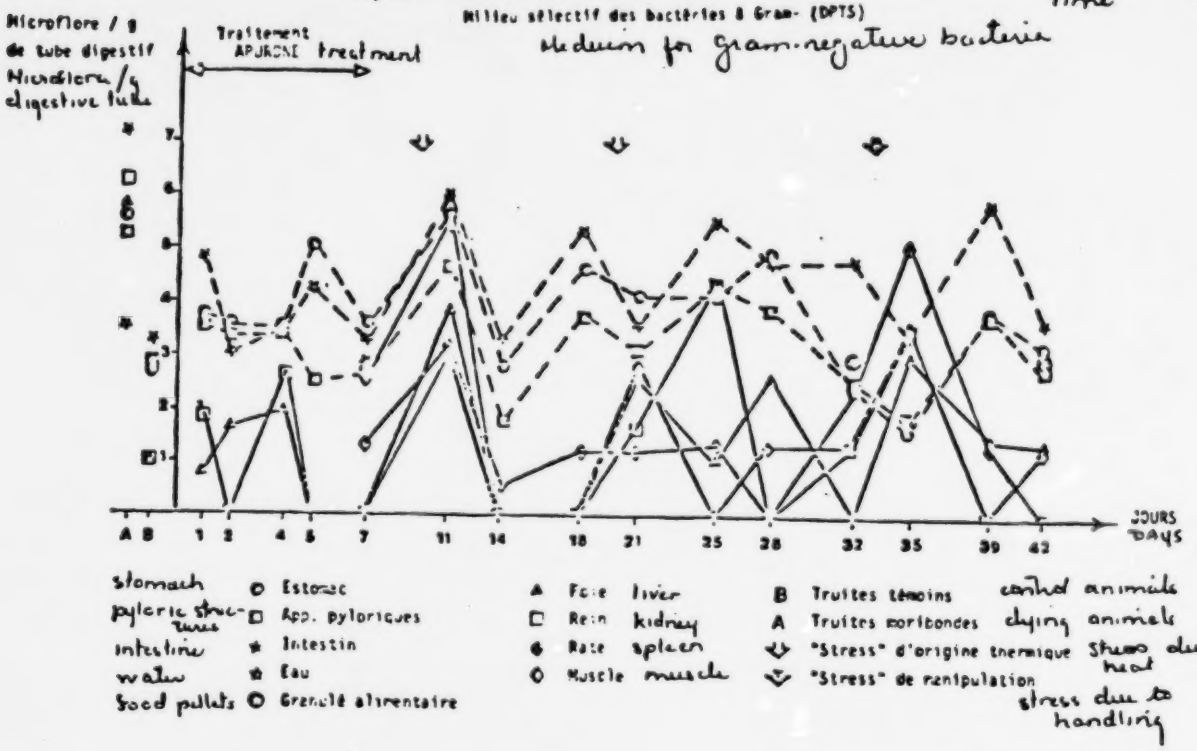
The constant presence of Hafnia alvei in the samples does not constitute evidence that the species remains permanently in trout organs. Since it is very common in aquatic environments, its presence may simply indicate that it continually passes through the digestive tract.

The disappearance of Yersinia ruckeri from the dominant flora following treatment indicates that the antibiotic was effective against the target organism. The re-emergence of the bacterium in organs that were in contact only with the subject's internal environ-

Floral count as a function of Time

Figure 1 : Evolution de la flore
Milieu sélectif des bactéries à Gram- (DPTS)

Medium for gram-negative bacteria

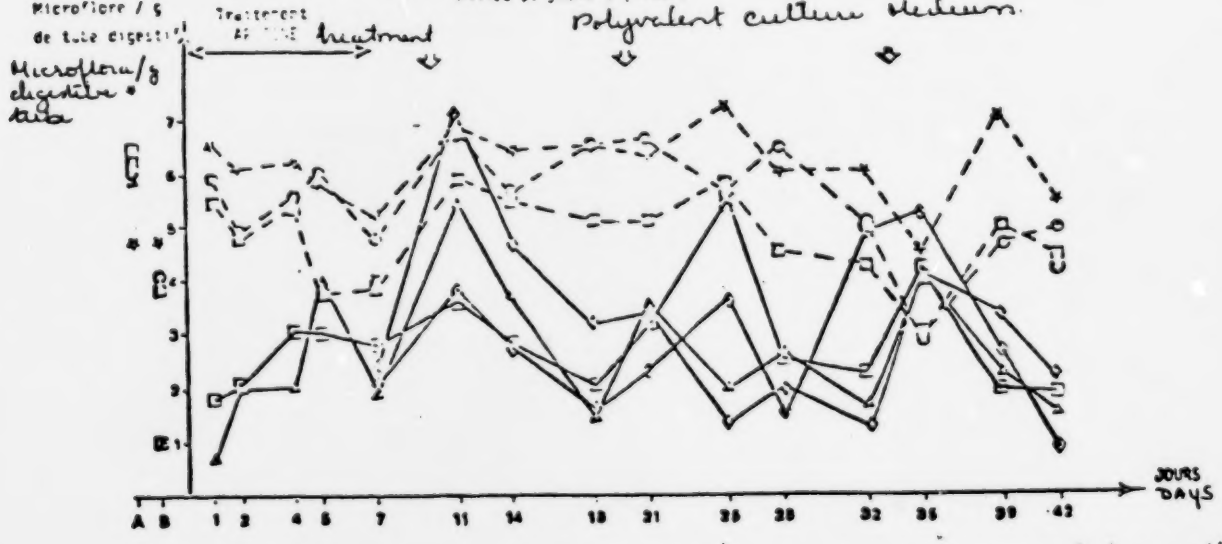

Microflore / g de tube digestif
Microflora/g digestive tube
Traitement APJRONE treatment
JOURS DAYS
A B 1 2 4 5 7 11 14 18 21 25 28 32 35 39 42

stomach	Estomac	Foie liver	Truites témoins	control animals
pyloric structures	App. pyloriques	Rein kidney	Truites moribondes	dying animals
intestine	Intestin	Rate spleen	"Stress" d'origine thermique	stress due to heat
water	Eau	Muscle muscle	"Stress" de manipulation	stress due to handling
food pellets	Granulé alimentaire			

Floral Count as a Function of Time

Figure 2 : Evolution de la flore
Milieu polyvalent (T.S.I.P.)

Polyvalent culture medium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Microflora/g digestive tube
Traitement treatment
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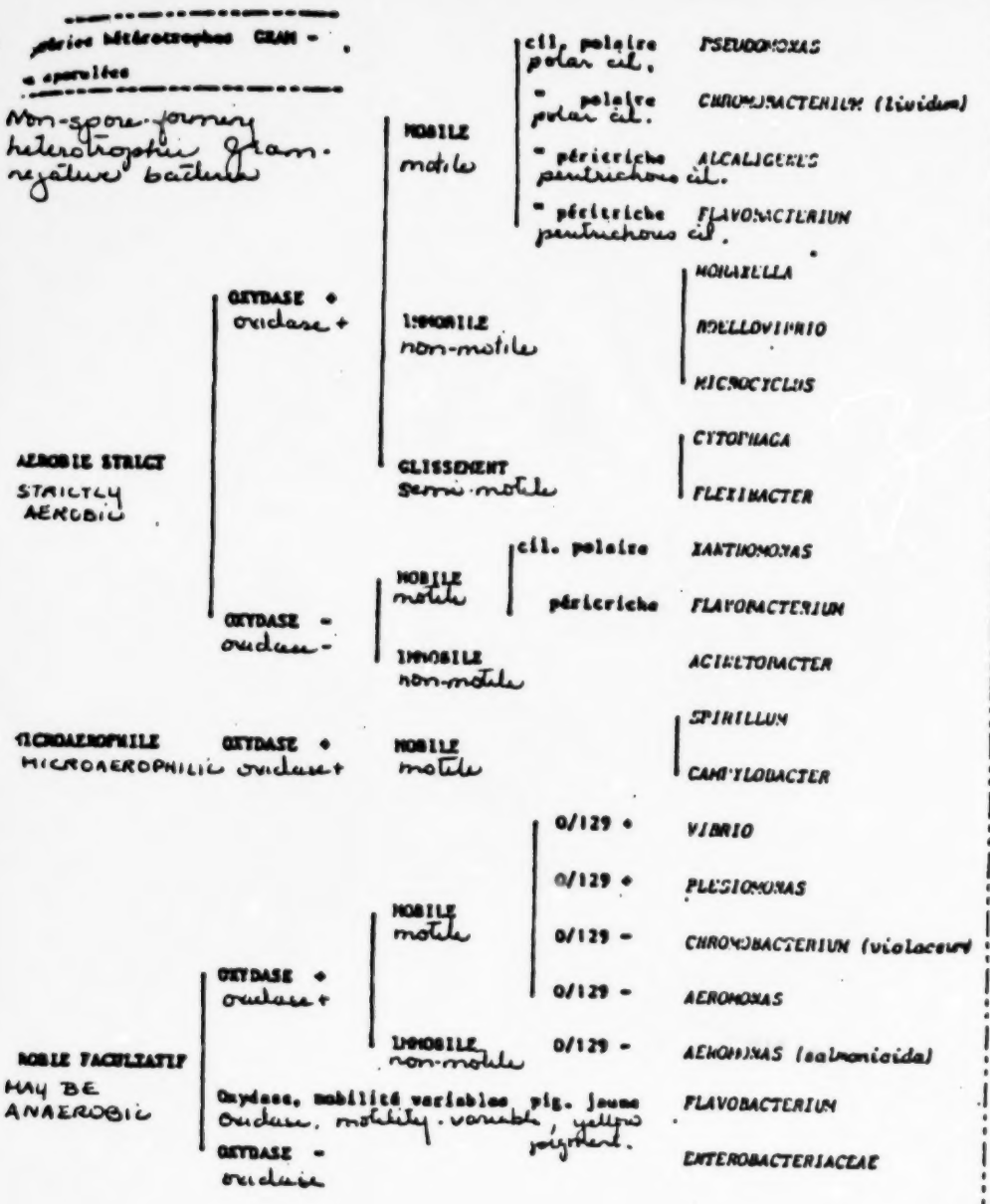
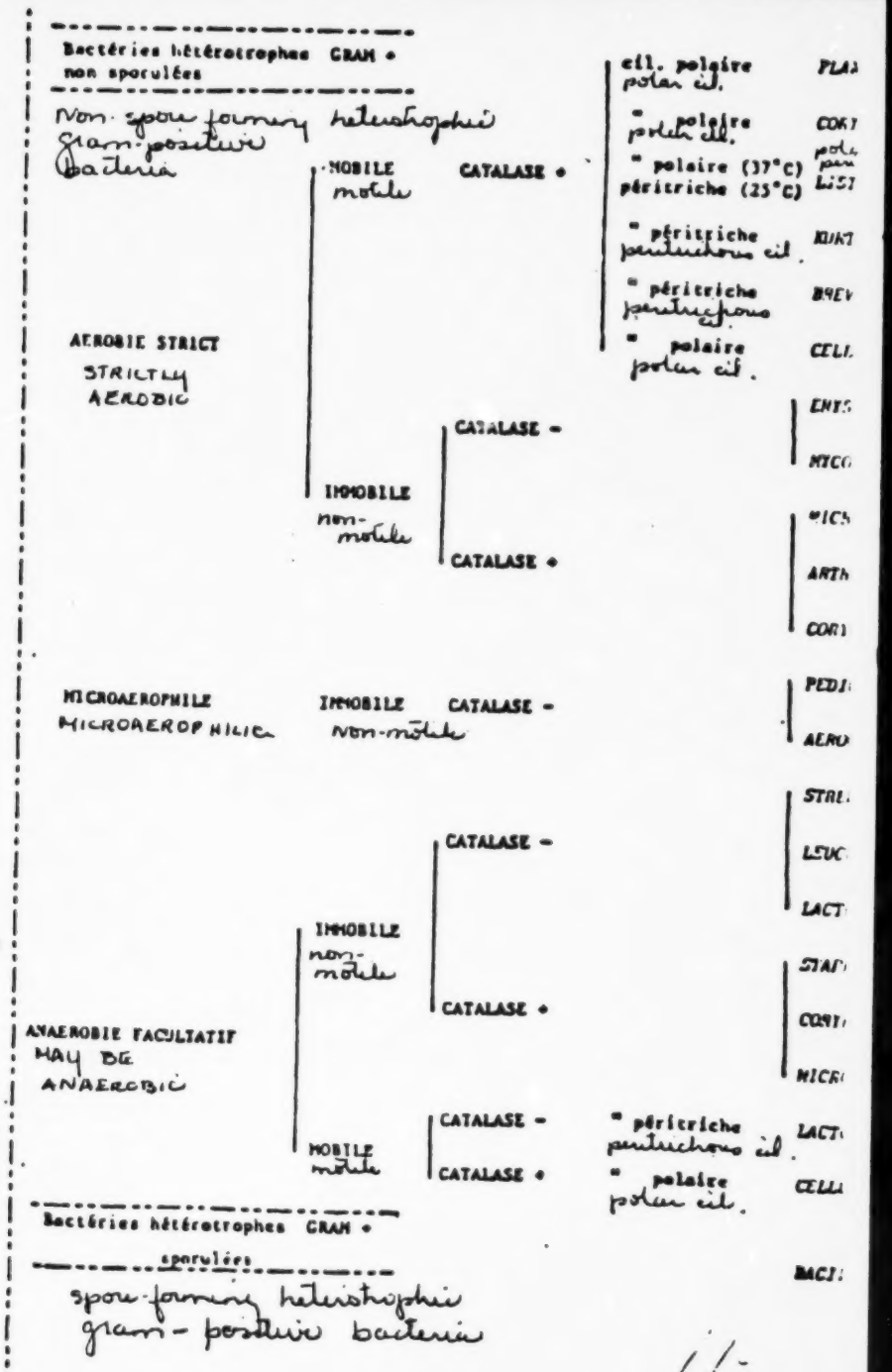


Tableau I - Schéma d'identification des isolats bactériens.
Table I Identification Chart of Bacterial Isolates



	Ensemble des compartiments digestifs Samples from the Digestive Compartments	Prélèvements autres que le tube digestif Samples from all other tissues
Sujets moribonds Dying subjects	*Hafnia alvei*	Foie, Rein : Liver, kidney : *Yersinia ruckeri*
Témoins Controls	*Hafnia alvei*, Corynébactéries	Foie : Liver : Corynébactérie, *Hafnia alvei*
	Aeromonas, Levures yeasts	Rein : Kidney *Aeromonas*, *Hafnia alvei*
Traitement antibiotique à l'APURONE (FLUMEQUINE) Antibiotic treatment with Apurone (Flumequine)		
7ème jour 7th day	———	Foie : Liver Levures, yeasts *Flavobacterium* Entérobactéries Enterobacteria
		Rein : Kidney *Hafnia alvei*, Levures, yeasts
		Rate et Muscle : Spleen + Muscle Levures yeasts
11ème jour 11th day	*Hafnia alvei*, *Aeromonas* *Enterobacter agglomerans* *Citrobacter*	Foie : Liver *Citrobacter*, *Hafnia alvei*, *Aeromonas*
		Rein : Kidney *Pseudomonas*, *Aeromonas* Corynébactéries Corynebacteria
		Rate : Spleen *Enterobacter agglomerans*, *Aeromonas* *Citrobacter freundii*
		Muscle : Muscle *Pseudomonas*
14ème jours 14th day	yeasts Levures, *Hafnia alvei*, *Aeromonas* *Enterobacter agglomerans*, *Pseudomonas*	Liver, Kidney Foie, Rein : Rate, Muscle Spleen Muscle Levures, Corynébactéries yeasts, Corynebacteria
18ème jour 18th day	———	Foie : Liver *Pseudomonas*
		Rate : Spleen *Pseudomonas*
		Rein : Kidney *Yersinia ruckeri*
21ème jour 21st day	*Hafnia alvei*, *Pseudomonas*, Levures yeasts *Aeromonas*, Corynébactéries corynebacteria	Foie : Liver *Hafnia alvei*, *Aeromonas*, *Yersinia ruckeri*, Corynébactéries Corynebacteria
		Rein : Kidney *Aeromonas*, Corynébactéries, Levures corynebacteria, yeasts
		Rate : Spleen Levures, yeasts *Aeromonas*
		Muscle : Muscle *Aeromonas*, *Hafnia alvei*, Levures, yeasts *Pseudomonas*
25ème jour 25th day	———	Rein : Kidney *Yersinia ruckeri*, *Hafnia alvei*
		Muscle : Muscle *Citrobacter freundii*
32ème jour 32nd day	*Hafnia alvei*, *Yersinia ruckeri*	Muscle : Muscle *Hafnia alvei*, *Aeromonas*
35ème jour 35th day	*Yersinia ruckeri*, *Hafnia alvei* *Aeromonas*, *Citrobacter freundii*	Foie : Liver *Yersinia ruckeri*
		Rein : Kidney *Yersinia ruckeri*
		Rate : Spleen *Yersinia ruckeri*
		Muscle : Muscle *Yersinia ruckeri*
42ème jour 42nd day	———	Foie : Liver *Pseudomonas*
		Rein : Kidney Corynébactéries, Levures, Citrobacter corynebacteria, yeasts
		Rate : Spleen *Yersinia ruckeri*, Corynébactéries corynebacteria

ment, then in the digestive compartments, suggests that the taxon does not remain in the digestive tract. In healthy specimens, in fact, it is found in the kidneys where it reproduces and from where it invades the other organs as a result of stress.

Our findings indicate that the presence of Yersinia ruckeri in concentrations of 10^6 and even 10^7 bacteria/gram of sample, exclusive of all other species, is not enough in itself to induce the development of clinical symptoms. A minimum number of other bacteria is needed for signs of Yersinia ruckeri activity to become manifest.

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